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FOLLOWING MICROWAVE EXPOSURE

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Research was conducted according to the principles enunciated in the
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ABSTRACT

Gamma-aminobutyric acid (GABA) metabolism was studied in rats chronically exposed to 2.86 GHz microwaves at an incident power level of 10 mW/cm^2 or acutely exposed to incident power levels of 40 or 80 mW/cm^2 . No changes occurred in whole brain GABA levels or L-glutamic decarboxylase activity following these exposures. These results suggest that an altered GABA metabolism is not involved in reported responses of the nervous system to microwave exposure.

I. INTRODUCTION

Animals chronically exposed to microwave radiation at relatively low power levels have been reported to show signs of both behavioral⁴ and neurochemical⁷ effects, and acute exposure to higher power levels has been reported to result in other neurologic manifestations, such as convulsions.^{1,3} The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and the enzyme which synthesizes GABA, L-glutamic decarboxylase (GAD), play an important role in regulation of neuronal activity in the brain.⁸ It is possible, therefore, that altered GABA metabolism is involved in the reported response of animals to microwave exposure. In view of the current controversy concerning biological effects of microwave exposure, and the sparsity of information on precise mechanisms involved, we feel it appropriate to report the results of our experiments, which indicate that alterations in GABA metabolism do not occur following exposure to microwave radiation.

II. MATERIALS AND METHODS

Microwave irradiation conditions. Male Sprague-Dawley rats from the AFRRRI colony were constrained in individual Plexiglas containers (3" x 3" x 7.5") in which holes had been drilled for ventilation. The individual containers were housed in a carrier so that the long axis of each container was perpendicular to the direction of propagation of the incident radiation. The rats were irradiated in groups of eight for the chronic exposures and singly for the acute exposures, with at least one empty container placed in the carrier directly above and below each group of rats.

The radiation source* produced 2.86 GHz microwave radiation with a pulse duration of 1 μ sec, a pulse repetition rate of 500 sec^{-1} , and 600 kW peak power (300 W average power). The source radiated into a lighted and ventilated screened chamber lined with anechoic material, through a waveguide transmission line and standard gain horn antenna. The animals in the container-carrier were placed in the chamber at a distance from the antenna where the incident power density was of the desired value. Power densities were measured with a Ramcor Model 1200 Densimeter† in place of the animals. In all exposures the animals were in the far field portion of the incident beam. The measured power densities agreed well with those calculated from the source output, antenna gain and the distance from the antenna.

Control rats for all experiments were treated identically to experimentals, including sham irradiation in a chamber similar in construction and adjacent to the exposure chamber. The temperatures of randomly selected animals were measured immediately upon removal of the animals from the exposure chamber, using a Digitec Model 251A (United Systems, Inc.) digital thermometer with a calibrated rectal probe. During the time between exposures both control and exposed animals were maintained in the exposure laboratory. Additional groups of rats of appropriate age and weight, housed in separate facilities, are referred to as unstressed controls.

Chronic exposures. In the first experiment, rats weighing 200-250 g were chronically exposed to incident power levels of 10 mW/cm^2 for 8 hours per day (8:00 a.m. to 4:00 p.m.) for either 3 or 5 days. On the day after the last day of irradiation,

* Manson Laboratory, Inc., Stamford, Connecticut, Model 275, 2.5 megawatt modulator, driving a Raytheon 4J31 pulse magnetron

† Serial No. D27-8, and receiving horn antenna Serial No. 27-8

approximately 18 hours after termination of the exposure, the rats were decapitated. The heads were immediately immersed in liquid nitrogen, with subsequent storage at -80°C until time of assay. In the second chronic exposure experiment, rats weighing 85-105 g were exposed to incident power levels of 10 mW/cm^2 for 4 hours per day (10:00 a.m. to 2:00 p.m.), 5 days per week (Monday through Friday) for either 4 or 8 weeks. These rats were decapitated immediately following the final exposure period, and the heads frozen and stored as described above.

Acute exposures. Acute exposures were conducted as described above except that the power output from the radiation source was increased and the distance from the antenna to the animals was decreased to provide exposure to incident power levels of either 40 or 80 mW/cm^2 for 20 or 5 minutes, respectively. The rats (250-300 g) were decapitated immediately after exposure, and the heads frozen and stored as in the chronic exposure experiments.

GABA assay. Rapid freezing of the rat heads in liquid nitrogen usually resulted in bilateral splitting of the skull and brain facilitating removal of symmetrical half-brain portions (600-700 mg) of frozen tissue. One of these portions was removed and GABA extracted as follows. A 10 percent (w/v) homogenate in 80 percent ethanol was centrifuged 20 minutes at $5000 \times g$, with the residue being washed twice with 80 percent ethanol. The combined supernates were evaporated under reduced pressure at 60°C overnight and the residue was resuspended in H_2O . This aqueous extract was then clarified by washing with CHCl_3 . GABA concentrations were then determined enzymatically by the method of Scott and Jakoby.⁶ Pseudomonas fluorescens A.T.C.C. 13430 was obtained from International Mining and Chemical Corporation, Skokie, Illinois. Whole

brain GABA concentrations are expressed as micromoles of GABA per gram wet weight of brain, and are presented as the average \pm standard error, with the number of samples in parentheses.

GAD assay. A 12.5 percent homogenate of each remaining half-brain was formed in a solution containing 50 mM potassium phosphate pH 6.8 and 1 mM EDTA potassium salt. After centrifugation for 20 minutes at 35,000 x g, GAD activity in the supernate was determined by an isotopic assay similar to that employed by Roberts and Simonsen⁵ as modified by Wilson et al.⁹ In an incubation volume of 0.05 ml, final concentrations were 50 mM potassium phosphate pH 6.8, 1 mM EDTA potassium salt, 0.5 mM pyridoxal phosphate, 0.5 percent Triton X-100, 1 mM β -mercaptoethanol, 1 mM GABA, and 5 mM L-glutamate (1-¹⁴C) specific activity 1.25 mCi/mmole (obtained from CalAtomic, Inc., Los Angeles, California). The ¹⁴CO₂ produced was collected and counted with 90 percent efficiency using a Nuclear-Chicago Mark II liquid scintillation spectrometer. GAD activity is expressed as micromoles of substrate converted per hour per gram wet weight of brain, and is presented as the average \pm standard error, with the number of samples in parentheses.

III. RESULTS

Chronic exposures. The rats chronically exposed to an incident power level of 10 mW/cm² showed only moderate signs of heat stress. During exposure, rectal temperatures (sampled randomly) did not rise significantly above those of control rats. There were no significant differences in whole brain GABA levels or GAD activity between the irradiated and appropriate unstressed control and sham irradiated rats after exposures of either 3 or 5 days or 4 or 8 weeks (Tables I and II).

Table I. Whole Brain GABA Levels in Rats Chronically
Exposed to Incident Power Levels of 10 mW/cm²
Microwave Radiation at 2.86 GHz

Duration of exposure	GABA (μ mole/g)		
	Unstressed controls	Sham controls	Exposed
8 hours/day			
3 days	2.09 \pm .08 (11)	2.17 \pm .08 (8)	2.18 \pm .08 (8)
5 days		2.08 \pm .10 (8)	2.12 \pm .08 (8)
4 hours/day			
4 weeks	2.27 \pm .04 (7)	2.30 \pm .11 (7)	2.32 \pm .08 (7)
8 weeks	*	2.42 \pm .11 (7)	2.40 \pm .14 (8)

* No unstressed controls were analyzed for the 8-week groups

Table II. Whole Brain GAD Activity in Rats Chronically
Exposed to Incident Power Levels of 10 mW/cm²
Microwave Radiation at 2.86 GHz

Duration of exposure	GAD activity (μ mole/g per h)		
	Unstressed controls	Sham controls	Exposed
8 hours/day			
3 days	10.7 \pm 0.5 (8)	8.9 \pm 0.7 (4)	9.3 \pm 0.9 (4)
5 days		11.7 \pm 0.3 (4)	11.6 \pm 0.4 (4)
4 hours/day			
4 weeks	12.4 \pm 0.8 (6)	10.5 \pm 0.6 (7)	10.2 \pm 0.7 (5)
8 weeks	*	12.9 \pm 0.7 (7)	11.1 \pm 0.4 (6)

* No unstressed controls were analyzed for the 8-week groups

Acute exposures. The rats exposed to incident power levels of 40 mW/cm² for 20 minutes showed signs of general hyperthermia, i.e., panting, salivation, increased defecation and urination, and fatigue. Those exposed to incident power levels of 80 mW/cm² showed similar but somewhat more severe symptoms. All rats remained conscious during these exposures and none showed signs of convulsions. Rectal temperatures were not obtained for these rats since they were killed immediately after exposure. However in other groups of rats similarly exposed to incident power levels of either 40 or 80 mW/cm², rectal temperatures rose no more than 3°C above the control level of 35.6°C. These acute exposures also had no apparent effect on whole brain GABA levels or GAD activity (Table III).

Table III. Whole Brain GABA Levels and GAD Activity in Rats Acutely Exposed to 2.86 GHz Microwave Radiation at Incident Power Levels of Either 40 or 80 mW/cm²

Treatment	GABA (μmole/g)	GAD (μmole/g per h)
Unstressed controls	2.27 ± 0.04 (7)	12.4 ± 0.8 (6)
Sham controls	2.31 ± 0.11 (4)	13.3 ± 1.2 (3)
40 mW/cm ² for 20 min	2.28 ± 0.07 (4)	12.1 ± 0.6 (4)
80 mW/cm ² for 5 min	2.28 ± 0.07 (8)	*

* GAD data not obtained

IV. DISCUSSION

The data presented in this report indicate that chronic exposure to incident power levels of 10 mW/cm² or acute exposure to incident power levels of 40 or 80 mW/cm² of 2.86 GHz microwave radiation does not alter normal GABA metabolism in rat brain.

Since biochemical determinations were conducted using whole brain homogenates we cannot rule out the possibility that the exposures induced regional metabolic alterations or impairment of the physiological role of GABA. However, whole brain determinations of both GABA levels and GAD activity have previously served as sensitive indicators of other central nervous system disorders.^{2, 10, 11} We conclude therefore that it is unlikely that alterations in GABA metabolism are involved in reported responses of the nervous system to microwave exposure.

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